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Chemical Composition and Active Antioxidants of *Eucommia ulmoides* Oliv. Bark

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Chemical composition of the *Eucommia ulmoides* bark, including extractives, proximate, mineral, fatty acid and monosaccharide compositions, was studied. The most abundant mineral was calcium (533.17 mg/100 g). α-linolenic acid (24.7%) and linoleic acid (24.3%), showed higher contents among the fatty acids. Major monosaccharides of *E. ulmoides* bark were arabinose (13.94 mg/g), xylose (18.91 mg/g) and glucose (119.7 mg/g). From the bark of *E. ulmoides*, four compounds were isolated and their structures were elucidated as caffeic acid (Ⅰ), kaempferol (Ⅱ), quercetin (Ⅲ) and isoquercitrin (Ⅳ) by spectroscopic analysis such as NMR and MS, including cellulose TLC and other chemical evidence such as hydrolyzation and acetylation. The antioxidant activities of four isolated compounds were evaluated by DPPH free radical scavenging, hydroxyl scavenging and reducing power assays. The results indicated that all the isolated compounds showed higher DPPH radical scavenging activity than α-tocopherol and BHT that were used as positive controls and these four compounds exhibited considerable reducing power and hydroxyl radical (OH) scavenging activity. Considering from the results above, it suggests that the *E. ulmoides* bark is a potential natural source of antioxidant material.

Key words: Antioxidant, Bark, Chemical composition, *Eucommia ulmoides* Oliv.

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INTRODUCTION

Free radicals have been complicated in a number of diseases due to the oxidative damage to DNA, lipids, and proteins and which can result in the failure of cellular functions.[1,2] However, dietary intake of antioxidant compounds will reduce oxidative damage.[3] Some synthetic antioxidant compounds show side effects, for example, butylated hydroxyanisole (BHA) has been reported to increase incidences of neoplasias.[4] Therefore, considerable effort has been directed at identifying safe and natural antioxidants that can protect against oxidative stress. The phenolic compounds, such as phenolic acids, flavonols and flavonol glucosides, commonly found in many plants are involved in many biological activities, including the chelation of metals, scavenging active oxygen species, and antioxidative activity.[5-7]

Eucommia ulmoides Oliv., the only species of the Eucommiaceae family, is a traditional medicinal material used in east Asia. *E. ulmoides* bark was used to alleviate fatigue and strengthen internal organs.[8] Recently, the tree has been used to treat hypertension and diabetes.[9,10] The present study was carried out with the aim of identifying active antioxidants from *E. ulmoides* bark. Also, its chemical composition is very important as the material of nutritional and pharmaceutical product. To date, however, literature data on chemical composition of *E. ulmoides* bark are very limited. Chemical composition of *E. ulmoides* bark was assayed in the present study.

MATERIALS AND METHODS

1. Materials

Bark of *E. ulmoides* was purchased from local herbal store (Tianjin, China). It was dried at room temperature and then ground to fine powder and kept in plastic containers at room temperature.

2. Chemical Composition Analysis

Chemical composition, including proximate, mineral, fatty acid and monosaccharide compositions was

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determined in triplicate. Proximate chemical composition analyses of the *E. ulmoides* bark for moisture, ash, crude protein (N×6.25) and crude fat, were determined according to AOAC official methods (AOAC, 2000).[11] Carbohydrate was determined by difference.

For mineral analysis, Na, K, Mg, Fe, and Zn contents were analyzed using Atomic Absorption Spectrophotometer (AnalytikjenaAG NOVA330, Germany). Calcium content was analyzed using nitrous oxide-acetylene gas after adding KCl to prevent the interfering of phosphorus by the AAS method. Phosphorus was assayed by UV/VIS Spectrophotometer DU800 (Beckman Coulter, USA) with Molybden blue method.

Monosaccharide content analysis was performed using the method of Blakeney et al.[12] The alditol acetates were extracted with dichloromethane and the organic phase was analyzed by GLC (Hewlett-Packard 5890 series II, USA) using DB-225 capillary column (30 m×0.25 mm, id×0.25 μm, df). The chromatographic conditions were the following: oven temperature: 235 ℃ (25 min); injector temperature: 285 ℃; detector temperature: 300 ℃ (FID).

Fatty acids methyl esters from the lipid extraction were analyzed by gas liquid chromatography using an Agilent 6890 N Gas chromatograph equipped with ZB-Wax capillary column (30 m×0.25 mm, id×0.25 μm, df). The chromatograph conditions were the following: initial temperature: 140 ℃ (3 min); program rate: 8 ℃/min; final temperature: 250 ℃; injector temperature: 250 ℃; FID (detector) temperature: 260 ℃.

3. Extraction, Isolation and Identification of Active Antioxidants

NMR spectra of the isolates were recorded in methanol-d_4 using a Bruker 400 spectrometer. Positive ion FAB-MS was done with a VG70-VSEQ (VG, Analitical, UK) mass spectrometer. TLC analysis was performed on 25 DC-Plastik-folien Cellulose F (Merk) plates and developed with *t*-buthanol-acetic acid-H_2O (3:1:1, v/v/v) and acetic acid-H_2O (3:47, v/v).

About 1 kg powdered *E. ulmoides* bark was extracted at room temperature with water, concentrated under reduced pressure and partitioned using *n*-hexane, CH_2Cl_2 and EtOAc, in sequence. After freeze dried, *n*-hexane (1.28 g), CH_2Cl_2 (890 mg), EtOAc (5.22 g) and H_2O (92.35 g) partitioned fractions were obtained. A portion of EtOAc partitioned fraction (4.70 g) was applied to a Sephadex LH-20 column and eluted with methanol (MeOH)-H_2O (3:1, v/v) until the eluents were almost colorless to give 7 fractions and labeled as EUE-1 (86 mg), EUE-2 (1.12 g), EUE-3 (2.17 g), EUE-4 (560 mg) and EUE-5 (144 mg), EUE-6 (557 mg) and EUE-7 (35 mg). Fraction EUE-2 was rechromatographed using MeOH-H_2O (1:1 and 1:3, v/v) to give 46 mg of compound I. Fraction EUE-3 was rechromatographed with MeOH-H_2O (1:1 and 1:3, v/v) and ethanol (EtOH)-H_2O (1:4, v/v) to give 28 mg of compound II and 21 mg of III. Fraction EUE-6 was also rechromatographed with by 50% aqueous MeOH followed by EtOH-hexane (4:1 and 2:1, v/v) to give 59 mg of compound IV.

4. Antioxidant Analysis

The scavenging activity of the stable DPPH free radical was determined by the method of Yoshida et al. with a slight modification.[13] MeOH solutions (4 ml) of samples at different concentrations (2~40 μg/ml) were added to a solution of DPPH (1.5×10^{-4} M, 1 ml) in MeOH. After mixing gently and standing at room temperature for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer (Libra S32, Biochrom LTD). The results were calculated by taking the mean of all triplicated values. BHT and α-tocopherol were used as positive controls. The DPPH radical scavenging activity was calculated according to the following equation:

Scavenging activity (%)=$[1-(A_1-A_2)/A_0]\times100\%$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without DPPH. IC_{50} values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH free radicals.

Hydroxyl radical scavenging was carried out according to the method of Chung et al. (1997).[14] The Fenton reaction mixture consisted of 200 μl of FeSO4·7H_2O (10 mM), EDTA (10 mM) and 2-deoxyribose (10 mM). Then, 200 μl sample and 1 ml of 0.1 M phosphate buffer (pH 7.4) were added to make up a total volume of 1.8 ml. Thereafter, 200 μl of 10 mM H_2O_2 was added and the reaction mixture was incubated at 37 ℃ for 4 h. After incubation, 1 ml of 2.8% TCA and 1 ml of 1% TBA were added and the mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged (5 min, 3000 rpm) and the absorbance was measured at 532 nm. Each assay was performed in triplicate. BHT and α-tocopherol were used as positive controls. The hydroxyl radical scavenging activity was calculated according to the following equation:

Scavenging activity (%)=$[1-(A_1-A_2)/A_0]\times100\%$

Where A_0 was the absorbance of the control (blank,

Table 1. Proximate composition of *E. ulmoides* bark

Proximate composition (%)	
Moisture	8.34
Ash	6.15
Crude protein	5.51
Crude fat	6.89
Carbohydrate	73.11

Table 2. Mineral contents of E. ulmoides bark

Mineral (mg/100g)	
Ca	533.17
K	270.66
Na	38.87
Mg	87.51
Zn	0.62
Fe	20.35
P	35.31

Table 3. Monosaccharide content of *E. ulmoides* bark

Monosaccharide	Arabinose	Xylose	Glucose
Content (mg/g)	13.94	18.91	119.70

without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without 2-deoxyribose.

The reducing power of samples was determined as Ordoñez *et al.*[15] Sample solution (1 ml) in MeOH was mixed with 2.5 ml of 0.2 M (pH 6.6) phosphate buffer and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$), then incubated at 50 ℃ for 20 min. Then 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture, followed by centrifugation at 3000 rpm for 15 min. the upper layer solution (2.5 ml) was mixed with an equal volume of water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$) and the absorbance was measured photometrically at 700 nm. The reducing power tests were run in triplicate. Increase in absorbance of the reaction indicated the reducing power of the samples. BHT and α-tocopherol were used as positive controls.

In this study, all the data were analyzed by using SPSS 11.5.

5. Statistical Analysis

The data was subjected to one-way analysis of variation (ANOVA) and the significance of difference between means was determined by Tukey's test ($p<0.05$) for multiple comparisons.

RESULTS AND DISCUSSION

1. Chemical Composition Analysis

The proximate composition of *E. ulmoides* bark is shown in Table 1 and the content of crude fat (6.89%) was quite high compared with common dried food.[16] As shown in Table 2, some critical minerals for human health were found in *E. ulmoides* bark, showing that the most abundant mineral was Ca and the least abundant mineral was Zn. Major monosaccharides of *E. ulmoides* bark were arabinose, xylose and glucose (Table 3), of which arabinose is a component sugar of a polysaccharide-*Eucomman* B which has activating effect on the reticuloendothelial system.[17] There were about 9 fatty acids in *E. ulmoides* bark, with 37.0% saturated and 63.0% unsaturated (Fig. 1). Of those fatty acids, the content of two unsaturated fatty acids, α-linolenic acid (18:3 α) and linoleic acid (18:2), was high, with content of 24.7% and 24.3%, respectively. α-linolenic acid is very useful to human and may treat many diseases such as cardiovascular disease, cancer and immune disorders.[18]

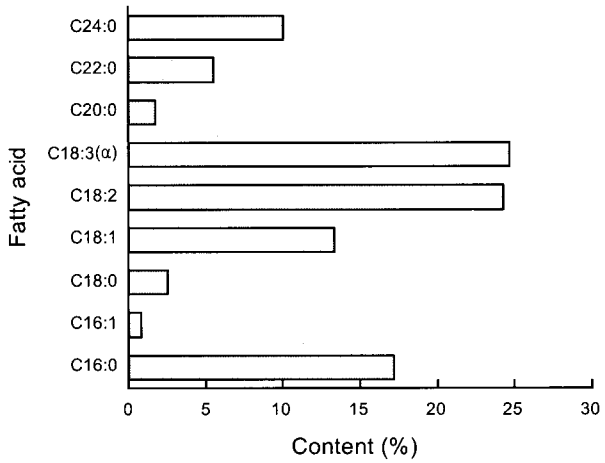

Fig. 1. Fatty acid content of *E. ulmoides* bark

2. Identification of Active Antioxidants

As shown in Fig. 2a and b, one phenolic acid, caffeic acid (I), two flavonols, kaempferol (II) and quercetin (III), and one flavonol glucoside, isoquercitrin (IV), were isolated from the EtOAc soluble fraction of *E. ulmoides* bark as yellowish powders which showed physical and spectral data virtually identical to those reported before.[19-24] Though they have been previously purified from *E. ulmoides* leaves,[25,26] to our knowledge, this was the first time of isolation of the four compounds from the bark of this species.

3. Antioxidant Activity

The antioxidant activities of four isolated compounds were determined by DPPH radical scavenging, hydroxyl scavenging and reducing power assays. The result, as

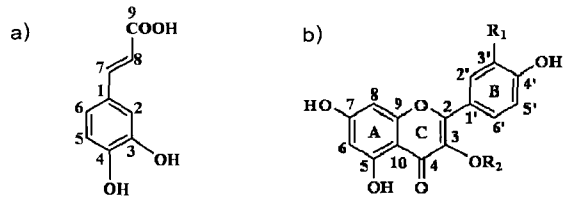

Fig. 2. Structure of compounds isolated from *E. ulmoides* bark
a) Caffeic acid (I)
b) R1=H, R2=H: kaempferol (II), R1=OH, R2=H: quercetin (III)
R_1=OH, R_2=glucose: isoquercitrin (IV)

Table 4. DPPH free radical scavenging activity (IC_{50} values) of the compounds isolated from *E. ulmoides* bark

	Samples	IC50 (μg/ml)
Positive controls	α-tocopherol	26
	BHT	30
Isolated compounds	Caffeic acid (I)	12
	Kaempferol (II)	14
	Quercetin (III)	11
	Isoquercitrin (IV)	22

shown in Table 4, showed that all the four compounds exhibited strong DPPH radical scavenging activity compared with reference compounds, α-tocopherol and BHT. Quercetin has the highest DPPH free radical scavenging activity, showing an IC_{50} of 11 μg/ml. The four compounds have been isolated from various plant materials, however, IC_{50} values by DPPH method showed certain variations due to test conditions in various studies. Kaempferol, quercetin and isoquercitrin from *Orostachy japonicus* showed IC_{50} as 1.87 μg/ml, 0.83 μg/ml and 0.77 μg/ml (IC_{50}=0.95 μg/ml for ascorbic acid as a control), respectively,[27] indicating that isoquercitrin showed higher antioxidant activity than kaempferol and quercetin. Caffeic acid from *Coleus aromaticus* showed an IC_{50} as 5.52 μg/ml.[28] All these results indicated that the four compounds have high DPPH radical scavenging activities. While hydroxyl scavenging assay revealed all the compounds from *E. ulmoides* bark showed considerable scavenging ability on hydroxyl (Fig. 3). Especially, caffeic acid at 10 μg/ml showed 84.4% scavenging activity whereas α-tocopherol and BHT at the same concentration showed 60.5% and 60.9% scavenging ability, respectively. The antioxidant activity has been reported to be concomitant with the reducing power.[29] In the present study, all the extractives showed great reducing power (Fig. 4). Considering the antioxidant assays above, it suggests that the four compounds from *E. ulmoides* bark have high antioxidant activities. High levels of free radicals, from both endogenous and exogenous sources, will create oxidative stress, which leads to a variety of damage to human. However,

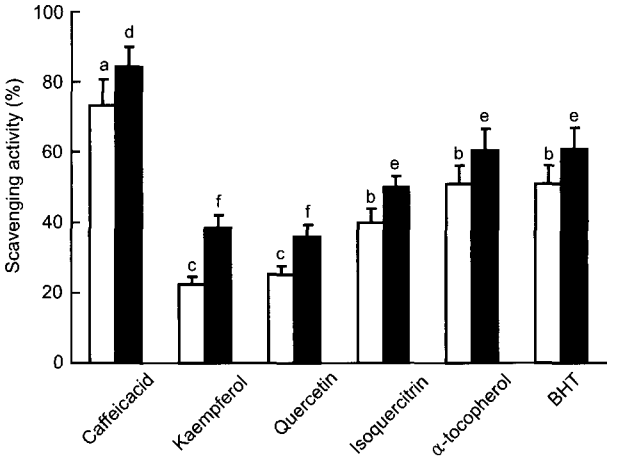

Fig. 3. Hydroxyl radical scavenging activity of the compounds isolated from *E. ulmoides* bark
Values are expressed as mean±SD (n=3)
a-c) columns with different letters are significantly (p<0.05) different when sample content was 1 μg/ml
d-f) columns with different letters are significantly (p<0.05) different when sample content was 10 μg/ml
BHT and α-tocopherol were used as positive controls.(□: 1 μg/ml, ■: 10 μg/ml)

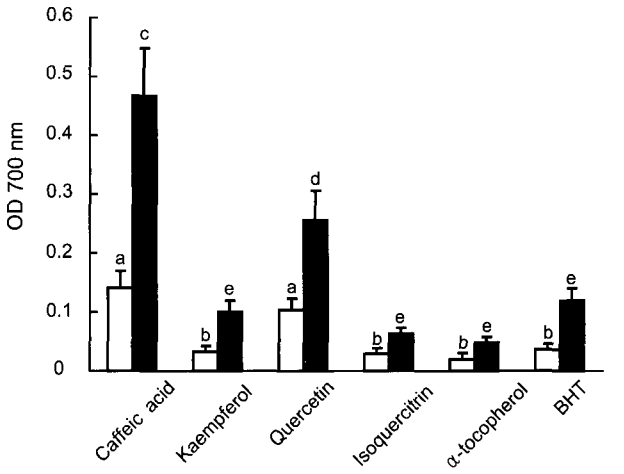

Fig. 4. Reducing power of the compounds isolated from *E. ulmoides* bark
Values are expressed as mean±SD (n=3)
a-b) columns with different letters are significantly (p<0.05) different when sample content was 5 μg/ml
c-e) columns with different letters are significantly (p<0.05) different when sample content was 15 μg/ml
BHT and α-tocopherol were used as positive controls(□: 5 μg/ml, ■: 15 μg/ml)

appropriate intake of natural food containing antioxidants will reduce oxidative damage. Our results suggest *E. ulmoides* bark contains some excellent antioxidants, indicating its value as potential source of natural antioxidants. Besides bark, leaves of *E. ulmoides* were found to have effective DPPH free radical-scavenging activity.[27] These results suggest that *E. ulmoides* can be used to treat some diseases associated with excess free radicals.

Literature Cited

1) Halliwell B, Gutteridge JM. Free radicals in biology and medicine, Oxford University press, Oxford, 1999
2) Chen FA, Wu AB, Shieh P, Kuo DH, Hsieh CY. Evaluation of the antioxidant activity of *Ruellia tuberose*. *Food Chem* 94:14-18, 2006
3) Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U.S.A.* 90:7915-7922, 1993
4) Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J Natl Cancer Inst* 70:343-352, 1983
5) Villaño D, Fernández-Pachón MS, Troncoso AM, García-Parrida MC. Influence of enological practices on the antioxidant activity of wines. *Food Chem* 95:394-404, 2006
6) Duan XJ, Zhang WW, Li XP, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga. *Polysiphonia ukceolata Food Chem* 95:37-43, 2006
7) Nara K, Miyoshi T, Honma T, Koga H. Antioxidative activity of bond-form phenolics in potato red. *Biosci Biotechnol Biochem* 70:1489-1491, 2006
8) Li ZL, Cui KM, Yuan ZD, Liu S. Regeneration of recovered bark in *Eucommia ulmoides*. *Chem Biol Agric Med Earth Sci* 26:33-40, 1983
9) Kwan CY, Zhang WB, Deyama T, Nishibe S. Endothelium-dependent vascular relaxation induced by *Eucommia ulmoides* Oliv. bark extract is mediated by NO and EDHF in small vessels. *Naunyn Schmiedebergs Arch Pharmacol* 369:206-211, 2004
10) Lee MK, Kim MJ, Cho SY, Park SA, Park KK, Jung UJ, Park HM, Choi MS. Hypoglycemic effect of Du-zhong (*Eucommia ulmoides* Oliv.) leaves in streptozotocin-induced diabetic rats. *Diabetes Res Clin Pract* 67:22-28, 2005
11) AOAC. Official methods of analysis of AOAC international, 17th edn. Gaitherburg, MD, Association of Official Analytical Chemists (AOAC) International, USA, 2000
12) Blakeney AB, Harris PJ, Henry RJ, Stone BA. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr Res* 113:291-299, 1983
13) Yoshida T, Mori K, Hatano T, Okumura T, Uehara I, Komagoe K, Fujita Y, Okuda T. Study on inhibition mechanism of autooxidation by tannins and flavonoids. V. Radical scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 37:1919-1921, 1989
14) Chung SK, Osawa T, Kawakishi S. Hydroxyl radical scavenging effects of spieces and scavenger from Brown Mustard (*Brassica nigra*). *Biosci Biotechnol Biochem* 61:118-123, 1997
15) Ordoñez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem* 97:452-458, 2006
16) Rural Nutrition Institute. Food composition table, 4th edn. Rural Development Administration. Korea, 1991
17) Tomoda M, Gonda R, Shimizu N, Kanari M. A reticuloendothelial system-activating glycan from the barks of *Eucommia ulmoides*. *Phytochemistry* 29:3091-3094, 1990
18) Kelley DS, Branch LB, Love JE, Taylor PC, Rivera YM, Iacono JM. Dietary alpha-linolenic acid and immunocompetence in humans. *Am J Clin Nutr* 53:40-46, 1991
19) Cheminat A, Zawatzky R, Becker H, Brouillard R. Caffeoyl conjugates from *Echinacea* species: Structures and biological activity. *Phytochemistry* 27:2787-2794, 1988
20) Markham KR, Ternai B, Stanly R, Geiger H, Mabry TJ. Carbon-13 NMR studies of flavonoids-III: Naturally occurring flavonoid glycosides and their acetylated derivatives. *Tetrahedron* 34:1389-1397, 1978
21) Okuyama T, Hosoyama K, Hiragam Y, Kurono G, Takemoto T. The constituents of *Osmunda spp*: A new flavonol glycoside of *Osmunda asiatica*. *Chem Pharm Bull* 26:3071-3074, 1978
22) Ternai B, Markham KR. Carbon-13 NMR studies of flavonoids-I: flavones and flavonols. *Tetrahedron* 32:565-569, 1976
23) Wenkert E, Gottlieb HE. Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds. *Phytochemistry* 16:1811-1816, 1977
24) Fernandez J, Reyes R, Ponce H, Oropeza M, Vancalsteren MR, Jankowski C, Campos MG. Isoquercitrin from Argemone platyceras inhibits carbachol and leukotriene D4-incuced contraction in guinea-pig airways. *Eur J Pharmacol* 522:108-115, 2005
25) Takeshi D, Sansei N, Yoshihisa N. Constituents and pharmacological effects of *Eucommia* and Siberian ginseng. *Acta Pharmacol Sin* 22:1057-1070, 2001
26) Kim HY, Moon BH, Lee HJ, Choi DH. Flavonol glycosides from the leaves of *Eucommia ulmoides* O. with glycation inhibitory activity. *J Ethnopharmacol* 93:227-230, 2004
27) Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, Park JC. Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylphdrazyl radical. *Phytomedicine* 10:544-551, 2003
28) Kumaran A, Karunakaran RJ. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chem* 100: 356-361, 2007
29) Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem* 73:285-290, 2001